(2) *Mobile phase.* Mix distilled water:glacial acetic acid:acetonitrile (800:10:190). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.0 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the baseline.

(d) *Preparation of working standard and sample solutions.* Use the working standard and sample solutions prepared as described in the individual monographs for the drug being tested.

(e) *Procedure.* Using the equipment, reagents, and operating conditions as described in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations.* Calculate the cefoxitin content as described in the individual monographs for the drug being tested.

[49 FR 47827, Dec. 7, 1984]

§ 436.348 High-pressure liquid chromatographic assay for ceforanide.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4-centimeter deflection;

(5) A suitable integrator; and

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed wth octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers to 10 micrometers in diameter, U.S.P. XX. A particular column used for analysis of ceforanide should not be used for the analysis of other drugs.—

(b) *Mobile phase.* Mix 18.0 milliliters of 10 percent aqueous tetrabutylammonium hydroxide and 8.56 milliliters of 11*N* potassium hydroxide. Add the mixture to approximately 700 milliliters of distilled water. Add 200 milliliters of reagent grade methanol. Adjust the pH of the mixture to pH 7.0 with concentrated phosphoric acid and dilute to 1,000 milliliters with distilled water. Prepare fresh daily. Filter the mobile phase through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard and sample solutions*—(1) *Preparation of working standard solution.* Prepare a solution containing 1,000 micrograms of ceforanide activity per milliliter in mobile phase. Inject working standard solution within 5 minutes after dissolution.

(2) *Preparation of sample solution.* Prepare the sample solution as directed in the individual monograph for the drug being tested. Inject sample solution within 5 minutes after dissolution.

(e) *Procedure.* Use the equipment, mobile phase, operating conditions, and working standard and sample solutions described in paragraphs (a), (b), (c), and (d) of this section, and proceed as directed in paragraph (e)(1) of this section.

(1) *System suitability test.* Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile

phase followed by three replicate injections of 10 microliters each of the working standard solution. Allow an elution time sufficient to obtain satisfactory separation of expected components after each injection. Record the peak responses and calculate the tailing factor, efficiency of the column, coefficient of variation, and capacity factor as described for system suitability tests in the U.S.P. XX General Chapter 621 chromatography. Proceed as directed in paragraph (e)(2) of this section if the following minimum performance requirements have been met:

(i) *Tailing factor.* The tailing factor is satisfactory if it is not more than 1.2;

(ii) *Efficiency of the column.* The efficiency of the column is satisfactory if it is greater than 1,900 theoretical plates;

(iii) *Coefficient of variation.* The coefficient of variation of at least three replicate injections is satisfactory if it is not more than 1.5 percent; and

(iv) *Capacity factor.* The capacity factor is satisfactory if it is not less than 1.8 and not more than 5.

If the minimum performance requirements are not met, adjustments must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (e)(2) of this section.

(2) *Determination of the chromatogram.* Inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations.* Calculate the ceforanide content as directed in the individual monograph for the drug being tested.

[49 FR 25846, June 25, 1984; 49 FR 34347, Aug. 30, 1984]

§ 436.349 High-pressure liquid chromatographic assay for L-lysine in ceforanide for injection.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A suitable pump capable of reproducibly delivering a liquid to a pressure of 5,000 pounds per square inch;

(2) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(3) A suitable recorder;

(4) A suitable integrator; and

(5) A 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers to 10 micrometers in diameter, U.S.P. XX.

(b) *Reagents*—(1) 2,4-Dinitrofluorobenzene solution. Weigh accurately approximately 760 milligrams of 2,4-dinitrofluorobenzene into a 50-milliliter volumetric flask. Dissolve and dilute to volume with absolute ethyl alcohol.

(2) Tris (hydroxymethyl) aminomethane (THAM) solution. Weigh accurately approximately 1.44 grams of THAM into a 100-milliliter volumetric flask. Dissolve and dilute to volume with distilled water.

(c) *Mobile phase.* Mix methanol and water (62:38), and adjust to pH 3.0 with glacial acetic acid.

(d) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.5 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the standard that is at least 50 percent of scale with a typical chart speed of 0.2 inch per minute.

(e) *Preparation of standard and sample solutions*—(1) *Preparation of standard solution.* Weigh accurately approximately 36 milligrams of *L*-lysine used as the standard into a 100-milliliter volumetric flask. Dissolve and dilute to volume with distilled water. Transfer 2.0 milliliters of the *L*-lysine solution into a 10-milliliter volumetric flask, add 2.0 milliliters of THAM solution and 3.0 milliliters of 2,4-dinitrofluorobenzene solution. Cap tightly and mix well. Place the flask in a 50° C water bath for 30 minutes. Remove from water bath, allow the flask to cool to room temperature, and dilute to volume with methanol. Mix well.

(2) *Preparation of sample solution.* Weigh accurately approximately 150